## REMARKS

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Claims 29-31, 41, 44, 46-52, and 59-63 are pending.[1] Claims 44, 62, and 63 are allowed. Examiner rejects claims 29-39, 41, 46-52, and 59-61 and objects to Claim 31. Applicant has canceled claim 31 without prejudice, making moot the objection thereto. Applicant has amended claim 48 to correct a typographical error. Applicant has amended claim 50 to omit reference to claim 47 thereby rendering moot the section 112 second paragraph rejection.

**CLAIM REJECTIONS UNDER 35 U.S.C. §103(a)**

Claims 29-39, 41, 46-52, and 59-61 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Chinese Patent No. 1,220,995 to Chen (hereinafter "Chen") in view of U.S. Patent No. 5,346,994 to Chomczyski (hereinafter "Chomczyski"). Examiner appears to overlook the substantial differences between the methods disclosed in Chen and the presently claimed invention. Briefly, the present application discloses two methods of purifying RNA from a sample. One claimed method utilizes phase separation and the other utilizes acidic phenol precipitation to selectively precipitate DNA, proteins, and other cellular debris while leaving RNA in a soluble form. Both these methods are significantly different thant the method disclosed in Chen.

"Rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated rational reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR Intern. Co. v. Teleflex Inc.* 127 S.Ct. 1727, 1741 (2007) (quoting *In re Kahn*, 441 F.3d 977, 988 (C.A.Fed. 2006). The Court further held that "apparent reason to combine the known elements in the fashion claimed

[1] Claims 1-28 are non-elected claims which are now the subject of a co-pending divisional application.

by the patent at issue" should be found in the prior art, the problem to be solved, or knowledge of skilled artisan. *Id.* at 1740-1741. Applicant respectfully submits that Examiner has failed to articulate "rational reasoning with some rational underpinning" to support his conclusion of obviousness and there was no apparent reason to combine the references.

Applicant's invention is concerned with purifying RNA from biological samples. Purity of the sample is defined and claimed as "RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR)." See claims 29, 44, 47, and 48. Examiner asserts that "Chen teaches a method for isolating purified RNA from a biological sample." Office Action page 4. However, the methods disclosed by Chen do not result in purified RNA. The methods in Chen result in isolated RNA of such low quality that at the end of the extraction process the protocol calls for the use of "RNA protectant" to dissolve the extracted RNA. RNA protectant functions to protect isolated RNA from degradation by contaminating enzymes. As further evidence that Chen does not result in isolation of sufficiently pure RNA, Applicant directs Examiner to his affidavit submitted on January 7, 2007, wherein it was shown that RNA isolated using the methods disclosed in Chen resulted in substantial DNA contamination. The lack of purity of the isolated RNA yielded by the Chen methods is undoubtedly due to the substantial differences in the methods disclosed in Chen and the presently claimed invention. One of the key differences being the presently claimed requirement of "a buffer at a concentration sufficient to maintain a pH in the [desired] range." See claims 29, 44, 47,and 48. Chen has no such buffer.

Thus, the novelty of the presently claimed invention is that substantially pure isolated RNA is obtained using a reagent buffered to maintain the pH in a specified range after the reagent is added to a sample. Specifically, the reagent is buffered to maintain a pH in a range "from about pH 3.6 to below pH 4.0" in claim 29 or "from about ph 3.6 to about pH 5.5" in

claims 47 and 48. Examiner acknowledges that "[w]hile Chen teaches the use of a pH adjusting component, Chen does not state that the amount used will be sufficient to maintain pH." Office Action page 7. Examiner relies on Chomczynski to provide the buffering element by stating that "Chomczynski teaches the use of a pH adjusting component in an RNA solvent solution where 'the solvent solution may include a buffering component . . . in an amount sufficient to maintain the pH of the solution.'" *Id.* Examiner then concludes that "[a]n ordinary practitioner would have been motivated to include sufficient buffering in the isolation buffer of Chen in order to maintain the pH since both Chen and Chomczynski teach and motivate the use of buffering components to maintain the pH of the solution." However, as discussed in greater detail below, Applicant respectfully asserts that this conclusion is not founded on a rational basis.

Chen actually discloses that the RNA extracting reagent may have a pH in the range of 3.5-6.5 buffered with a very low concentration of "pH regulator" (0.005%-0.02%). Chen does not disclose that that mixing the RNA extracting reagent with a biological sample results in the presently claimed pH "in the range from about pH 3.6 to below pH 4.0," (claim 29) or "from about ph 3.6 to about pH 5.5" (claims 47 and 48). Nor is it apparent that Chen even contemplated the importance of maintaining the pH of the mixture of the reagent and sample below pH 4. As is apparent by the miniscule concentration of buffering salts utilized in the Chen reagent (0.005%-0.02% concentration range of pH regulator), Chen did not contemplate maintaining the pH of the mixture of the reagent and tissue sample, much less maintaining the pH in the presently claimed ranges. It should be noted that most biological samples have a pH above 7, and are themselves buffered to maintain a neutral pH. Thus, very low concentrations of pH regulator, as disclosed in Chen, would provide virtually no buffering capacity, much less the buffering capacity to acidify a biological sample. Thus, Applicant respectfully asserts that Chen

does not motivate the use of buffering components to maintain the pH of the solution when mixed with a biological sample.

Chomczynski fails to provide the motivation lacking in Chen. Chomczynski is concerned with a shelf-stable solvent for isolating RNA, DNA, and proteins. *See* Chomczynski abstract. Chomczynski discloses a solvent solution of phenol and a guanidinium compound, and that the "solvent solution may include a buffering component, such as sodium acetate or sodium citrate, in an amount sufficient to maintain the pH of the solution in the range of about 4-6." Chomczynski fails to disclose an RNA isolation reagent as presently claimed or one having a buffering component capable of maintaining the presently claimed pH range of the reagent when mixed with a biological sample. Further, Chomczynski fails to contemplate that maintaining the pH of the mixture of biological sample and reagent below pH 4 will improve purification of RNA from the biological sample.

For at least the reasons present above, Applicant respectfully asserts that neither Chen nor Chomczynski contemplated that the presently claimed pH range would improve the purification of RNA from a biological sample, and therefore provide no motivation to the skilled artisan for their combination. Further, the skilled artisan, at the time of the invention, was not aware that the presently claimed pH range would improve the purification of RNA from a biological sample. Examiner has failed to provide a rational basis to support his conclusion that the skilled artisan would have been motivated to combine Chen and Chomczynski. Thus, at the time of the invention, no apparent reason existed for the combination of Chen with Chomczynski.

Applicant further asserts that Examiner misunderstands the evidence presented in the Applicant's affidavit submitted on January 17, 2007. In the affidavit, Applicant demonstrated that an embodiment of the presently claimed invention wherein the RNA extracting reagent had a

buffered pH of 3.8 was superior to the Chen RNA extracting reagent having an essentially unbuffered pH of 3.5. These data demonstrate that the key to the present invention is not the starting pH of the RNA extracting reagent, but rather that, as claimed, the reagent is buffered to maintain a pH in a specified range ("from about pH 3.6 to below pH 4.0" in claim 29 and "from about ph 3.6 to about pH 5.5" in claims 47 and 48) once the reagent is mixed with a sample. As described by Applicant in his affidavit on January 17, 2007, Chen recites a reagent having "0.005-0.02% pH regulator [which] corresponds to a maximal concentration of about 2 mM sodium ions available as a buffer. This provides almost no buffering capacity." Applicant's Affidavit paragraph 13. In contrast, Applicant's reagent contains in the 50 mM to 100 mM range of buffer as determined by calculations based on the examples in the specification. As Applicant has clearly shown, the methods of Chen result in substantial contamination with DNA presumably due to the substantial differences in the methods, and more particularly to the lack of buffer sufficient to maintain the pH of the solution. Thus, the affidavit provides evidence of the importance of buffering the reagent to maintain the pH as well as the unexpected result that such buffering results in purer isolated RNA.

Applicant respectfully submits that the arguments presented above are sufficient to overcome the obviousness rejections of the claims. However, additional arguments are presented below as to the patentability of certain claims over Chen.

Turning now to Examiner's rejection of claim 47, this claim requires the steps of:

> a) treating the sample with a mono-phase reagent comprising phenol at a final concentration ranging from about 3%$^{w/w}$ to less than 30%$^{w/w}$ and a buffer sufficient to maintain a pH of the composition in the range from about pH 3.6 to about pH 5.5,
> b) sedimenting or filtering the sample to obtain a purified sample substantially free of DNA, proteins, and cellular components without performing phase separation,
> c) adding to the purified sample about an equal volume of a water-soluble organic solvent to precipitate purified RNA which is RNA that does not reveal the

> presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR),
> d) sedimenting or filtering the precipitated RNA, and
> e) washing and solubilizing the precipitated RNA.

Thus, claim 47 is a mono-phase method of purifying RNA from a biological sample while expressly excluding a phase separation step during the sedimentation or filtration of DNA, proteins, and cellular components. Examiner rejects this claim stating that:

> Chen teaches the steps of: a) treatment with the monophase reagent comprising phenol in concentrations from 12-46% w/w with a pH from 3.5-6.5 and a chaotrope where guanidine isothiacyanate is used; b) sedimenting the sample to obtain a purified sample substantially free of DNA, proteins and cellular components; c) adding to the purified sample about an equal volume of a water soluble organic solvent to precipitate the purified RNA; d0 sedimenting the precipitated RNA; e) washing and solubilizing the precipitated RNA.

Office Action page 6. However, Applicant respectfully submits that Examiner mischaracterizes the disclosure in Chen. Chen actually discloses that:

> (1) The total RNA extractant is added to the tissue used for extraction of RNA in the proportion 1 ml to e.g. 50-100 mg of bulk tissue (homogenized on ice) or 10 cm2 of adherent culture cells or 105-107 suspended cells, mixed therewith, and then transferred to an Eppendorf tube.
> (2) A mixture of 0.1 ml of chloroform and isopentanol [chloroform:isopentanol = (24-49):1] is added and extraction is performed by shaking for 10 seconds.
> (3) The mixture is centrifuged . . ..
> (4) The upper layer of aqueous phase is transferred to a fresh Eppendorf tube (care being taken not to aspirate the interface).
> (5) An equal volume of isopropanol precooled to -20 C – -80 C is added and mixed by inversion for 10 sec.
> (6) The mixture is centrifuged . . ..
> (7) The supernatant is removed to obtain a white supernatant of total RNA.
> (8) The RNA sediment is washed with 1 ml of 75% ethanol.
> (9) The ethanol is discarded and the material is air dried or vacuum dried for 5 min.
> (10) The RNA is dissolved with the addition of a suitable amount of RNA protective agent and stored at low temperature pending use (underlining for emphasis).

Examiner's translation of Chen pages 3-4. Chen clearly discloses a phase separation by the addition of chloroform and isopentanol prior to the precipitation of DNA, protein, and cell

components. Thus, Chen does not disclose a monophase reagent for sedimenting a biological sample as Examiner states and does not read on claim 47.

Turning now to the rejection of claim 48. Claim 48 is a combination of the monophase purification as in claim 47 and the phase separation method of claim 29. Claim 48 requires:

> a) treating the sample with a mono-phase reagent comprising phenol at a final concentration ranging from about 3%$^{w/w}$ to less than 30%$^{w/w}$, at least one chaotrope, and a buffer sufficient to maintain a pH of the composition in the range from about pH 3.6 to about pH 5.5,
> b) sedimenting or filtering the sample to obtain a purified sample substantially free of DNA, proteins, and cellular components without performing phase separation,
> c) adding to the purified sample at least one hydrophobic organic solvent and a buffer in a concentration sufficient to maintain a pH of the purified sample in the range from about pH 3.6 to below pH 4.0,
> d) recovering purified RNA from an aqueous phase to which about an equal volume of a water soluble organic solvent is added to precipitate purified RNA which is RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR),
> e) sedimenting or filtrating the precipitated RNA, and
> f) washing and solubilizing the precipitated RNA.

Thus, the method of claim 48 requires that DNA, proteins, and cellular component be sedimented or filtered during a step that expressly excludes a phase separation step. This claim stands in contrast to the disclosure of Chen described above, which requires a phase separation step using the addition of chloroform and isopentanol prior to the precipitation of the contaminants. Thus, Chen does not read on claim 48.

Turning now to the rejection of claim 59, this claim requires the selective precipitation of higher molecular weight RNA from the purified sample. Selective precipitation is accomplished by precipitating the higher molecular weight RNA with "at least one water-soluble organic solvent at a concentration from about 10% $^{w/w}$ to about 40% $^{w/w}$." Claim 59. The key to this claim is that lower molecular weight RNA is not precipitated when the soluble

organic solvent is in the claimed range. Chen discloses precipitating isolated RNA with "[a]n equal volume of isopropanol," which results in a 50% concentration of solvent and the precipitation of high and low molecular weight RNA. Thus, Chen clearly does not disclose the presently claimed invention. Further, Chen does not contemplate the selective precipitation of higher molecular weight RNA, and thus provides no motivation to modify the disclosure to read on the presently claimed invention.

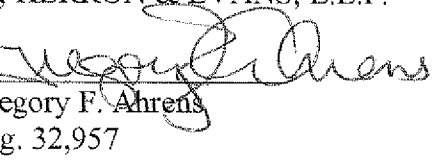
For at least these reasons, Applicant respectfully submits that the obviousness rejections have been overcome and should be withdrawn.

**Conclusion**

As a result of the remarks given herein, Applicant submits that the rejections of the pending claims have been overcome. Therefore, Applicant respectfully submits that this case is in condition for allowance and requests allowance of the pending claims.

If Examiner believes any detailed language of the claims requires further discussion, Examiner is respectfully asked to telephone the undersigned attorney so that the matter may be promptly resolved. Applicants also have submitted all fees believed to be necessary herewith. Should any additional fees or surcharges be deemed necessary, Examiner has authorization to charge fees or credit any overpayment to Deposit Account No. 23-3000.

Respectfully submitted,
WOOD, HERRON & EVANS, L.L.P.

By 
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